

Figure 1

gi 4502067 Human	GPVPTPPDNIQVQENFNISRIYKWKYNLAIGSTCPWLKKIMDRMTVSTLV	50
gi 197102775 Orangutan	GPVPTPPDNIQVQENFNISRIYKWKYNLAIGSTCPWLKKIMDRMTVSTLV	50
gi 403266141 SQ Monkey	SPVPTPEGIQVQENFNLSRIYKWKYNLAIGSTCPWLKKIMDRMTVSTLV	50
gi 311703 Mouse	DPASTLP-DIQVQENFSESRIYKWKYNLAIGSTCPWLKRIKDRMSVQTLV	49
gi 655878696 Rabbit	DPVPTLPDDIQVQENFELSRIYKWKYNLAIGSTCPWLKRIKDRMAVSTLV	50
gi 512843527 nm Rat	NPVPMFPDNIQVQENFDESRIYKWFNLAIGSTCPWLKRIKDRMSVSTLV	50
gi 472354701 Walrus	SPVLTTPDAIQVQENFDISRIYKWFNLAIGSTCPWLKRIKDRMSVSTLV	50
gi 471363267 Manatee	SPVKTPLNDIQVQENFDLPRYKWFNLAIGSTCPWLKRIKDRMSVSTLV	50
gi 478732996 Chicken	TPVGDQDEDIQVQENFEPERIKWKYDVAIGSTCPWLKRIKDRMSVSTLV	50
gi 556972695 Coelacanth	-GSPLRDEDIQVQENFDLPRYKWFNLAIGSTCPWLKRIKDRMSVSTLV	49
gi 147905774 Frog	CSPIQPEDNIQVQENFDLPRYKWFNLAIGSTCPWLKRIKDRMSVSTLV	50
gi 1091607 Plaice	-LPVLPPELYPTQENFDLPRYKWFNLAIGSTCPWLKRIKDRMSVSTLV	49
gi 4502067 Human	LGEGATEAEISMTSTRWRKGVCEETSGAYEKDITDGNFLYHNSKWNITME	100
gi 197102775 Orangutan	LGEGATEAEISMTSTRWRKGVCEETSGAYEKDITDGNFLYHNSKWNITME	100
gi 403266141 SQ Monkey	LEEGATEAEISMTSTRWRKGVCEETSGAYEKDITDGNFLYHNSKWNITME	100
gi 311703 Mouse	LQEGATEAEISMTSTRWRKGVCEETSGAYEKDITDGNFLYHNSKWNITME	99
gi 655878696 Rabbit	LGEGTETEISMTSTRWRKGVCEETSGAYEKDITDGNFLYHNSKWNITME	100
gi 512843527 nm Rat	LKGTTTETQISTTHWRKGVCEETSGAYEKDITDGNFLYHNSKWNITME	100
gi 472354701 Walrus	LGEGATDEISMTSTRWRKGVCEETSGAYEKDITDGNFLYHNSKWNITME	100
gi 471363267 Manatee	LGEGATDEISMTSTRWRKGVCEETSGAYEKDITDGNFLYHNSKWNITME	100
gi 478732996 Chicken	LGPGPSADQISTSTRWRKGVCEETSGAYEKDITDGNFLYHNSKWNITME	100
gi 556972695 Coelacanth	LQEGEQSDRISTSTRWRKGVCEETSGAYEKDITDGNFLYHNSKWNITME	99
gi 147905774 Frog	LSDGETDGEVRIVNTRMRHGTCSQIVGSYQKTTETPGKFDYFNARWGTTIQ	100
gi 1091607 Plaice	LEK-DTGNNKLVTRTLRHRGTCSQIVGSYQKTTETPGKFDYFNARWGTTIQ	98
gi 4502067 Human	SYVVHTNYDEYAIFLTNNFSSRRHGPTITAKLYGRAPQLRETLLQDFRVVA	150
gi 197102775 Orangutan	SYVVHTNYDEYAIFLTNNFSSRRHGPTITAKLYGRAPQLRETLLQDFRVVA	150
gi 403266141 SQ Monkey	SYVAHTNYDEYAIFLTNNFSSRRHGPTITAKLYGRAPQLRETLLQDFRVVA	150
gi 311703 Mouse	SYVVHTNYDEYAIFLTNNFSSRRHGPTITAKLYGRAPQLRETLLQDFRVVA	149
gi 655878696 Rabbit	SYVVHTNYDEYAIFLTNNFSSRRHGPTITAKLYGRAPQLRETLLQDFRVVA	150
gi 512843527 nm Rat	SYVVHTNYDEYAIFLTNNFSSRRHGPTITAKLYGRAPQLRETLLQDFRVVA	150
gi 472354701 Walrus	SYVVHTNYDEYAIFLTNNFSSRRHGPTITAKLYGRAPQLRETLLQDFRVVA	150
gi 471363267 Manatee	SYVVHTNYDEYAIFLTNNFSSRRHGPTITAKLYGRAPQLRETLLQDFRVVA	149
gi 478732996 Chicken	SYVLRNTYDEYAVILMKNKTSN-FGPTTTTLNLYGRAPQLRETLLQDFRVVA	149
gi 556972695 Coelacanth	SYVVHTNYDEYAIFLTNNFSSRRHGPTITAKLYGRAPQLRETLLQDFRVVA	148
gi 147905774 Frog	NYIVFTNYDEYVIMQMKKKG-SETTTTTLNLYGRAPQLRETLLQDFRVVA	149
gi 1091607 Plaice	AYVVHTNYDEYAIIMSKQNTSGENSTSLNLYSRTMSVRDVTLDDEFTLV	148
gi 4502067 Human	QGVGIPEDSIFTMADRGECPVGEQEPEPILIPR---	183 identity:100%
gi 197102775 Orangutan	QGVGIPEDSIFTMADRGECPVGEQEPEPILIPR---	183 99.5%
gi 403266141 SQ Monkey	QGVGIPEDSIFTMADRGECPVGEQEPEPILIPR---	183 98.5%
gi 311703 Mouse	LNVGISENSIIFMDRGECPVGEQEPEPILIPR---	182 75.8%
gi 655878696 Rabbit	LGVGIPEDSIFTMADRGECPVGEQEPEPILIPR---	183 82.1%
gi 512843527 nm Rat	LGVGIPEDSIFTMADRGECPVGEQEPEPILIPR---	183 77.6%
gi 472354701 Walrus	LGVGIPEDSIFTMADRGECPVGEQEPEPILIPR---	183 81.4%
gi 471363267 Manatee	LGVGIPEDSIFTMADRGECPVGEQEPEPILIPR---	182 76.4%
gi 478732996 Chicken	LEMGIADSVFILANKGECPVGEQEPEPILIPR---	179 50.3%
gi 556972695 Coelacanth	LDQGIEDSIVILPKRDECPVGEQEPEPILIPR---	180 50.0%
gi 147905774 Frog	LAQGIEDSIVILPKRDECPVGEQEPEPILIPR---	179 48.0%
gi 1091607 Plaice	RHQMSDDTIIIKQNKGDIPGEQEPEPILIPR---	184 36.5%

Figure 2

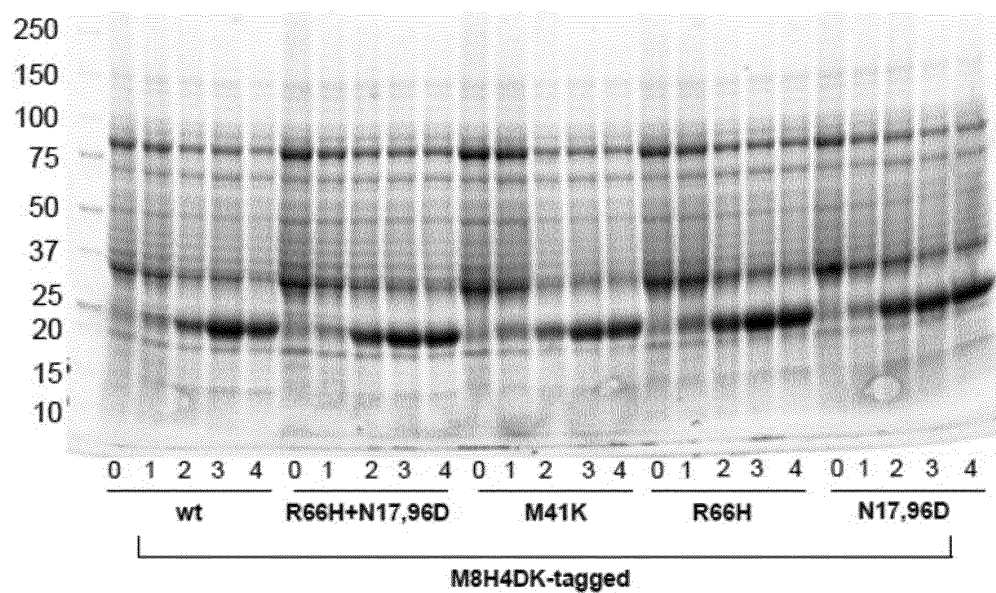
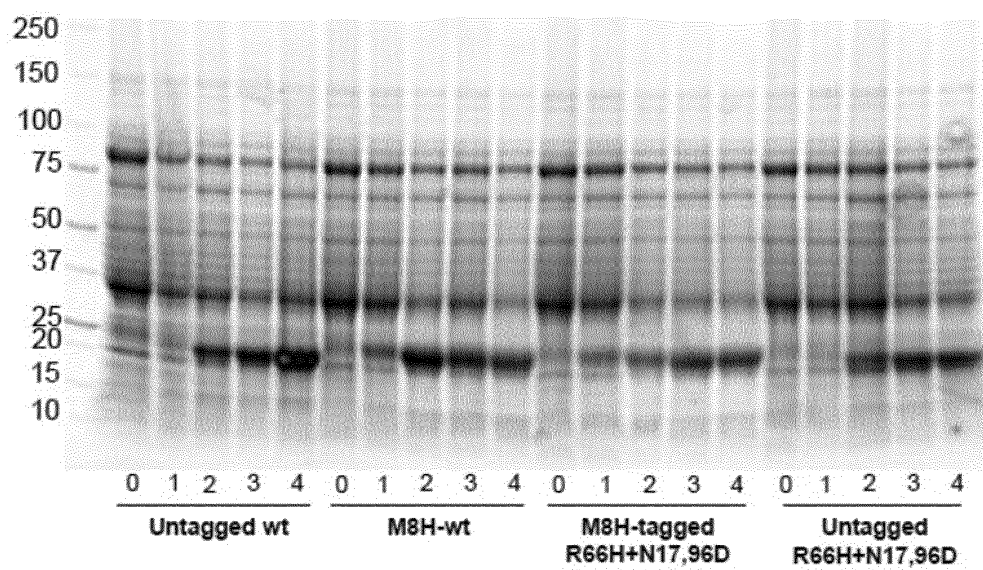


Figure 3

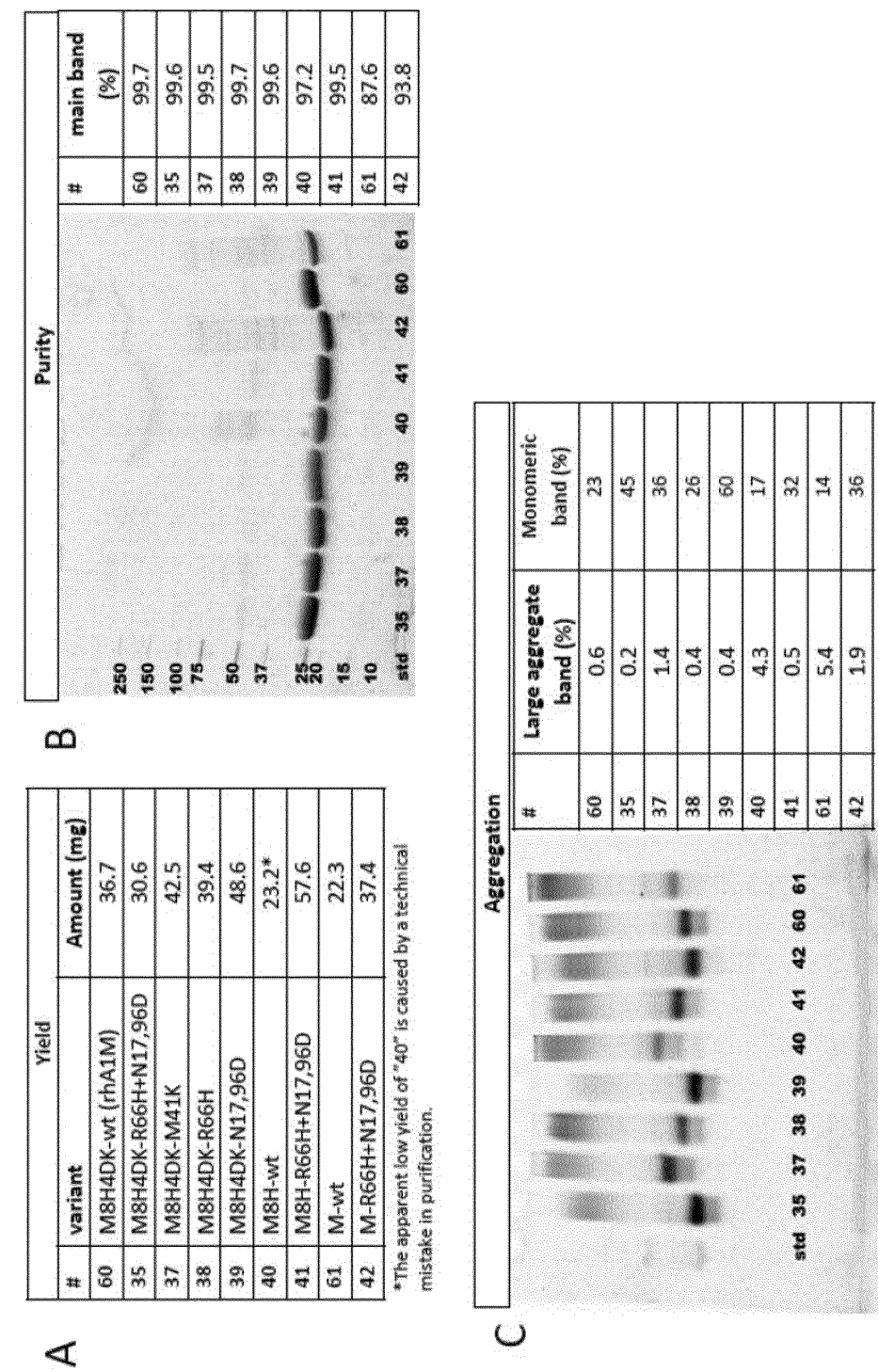


Figure 4

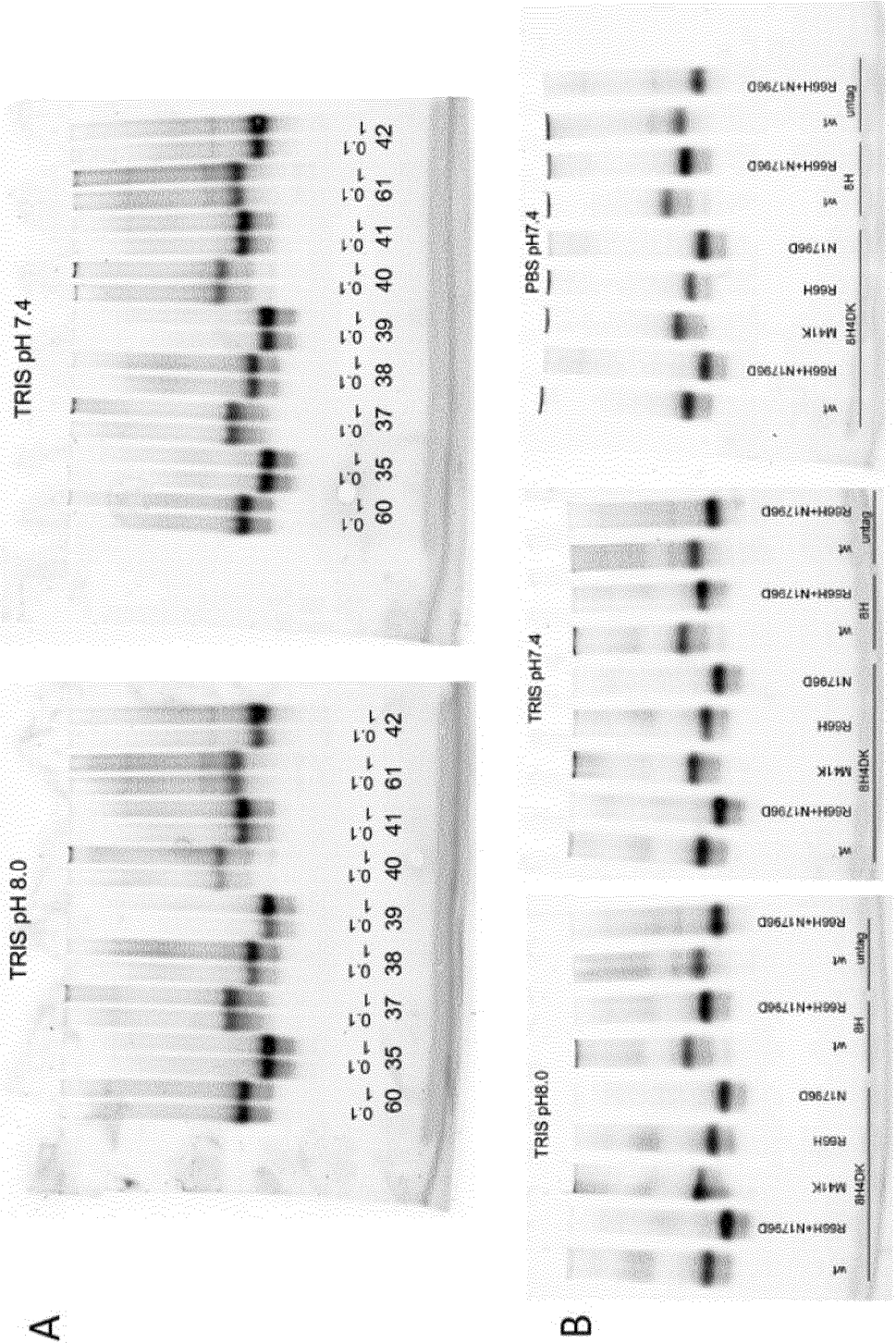


Figure 5

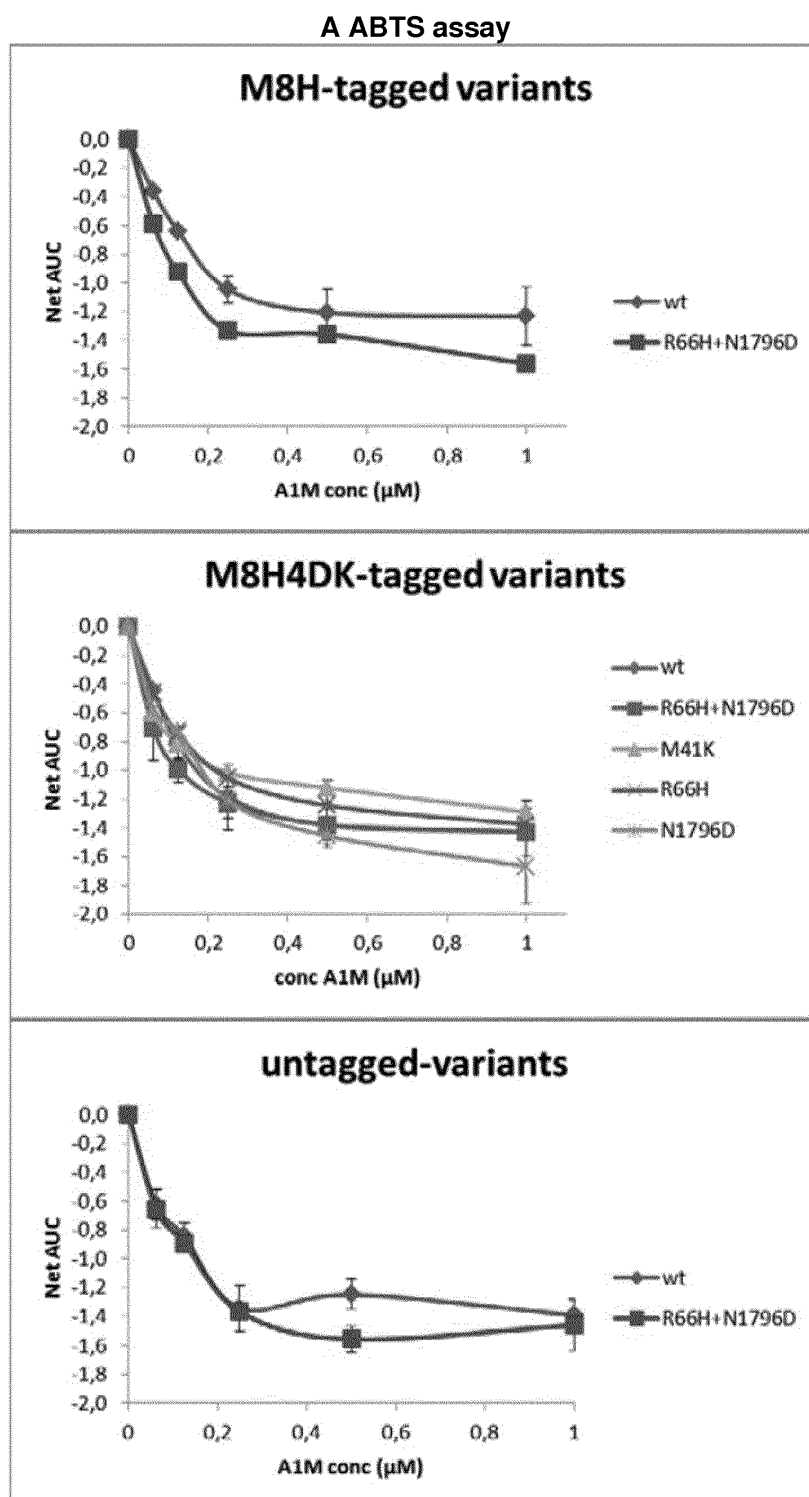


Figure 5 – continued

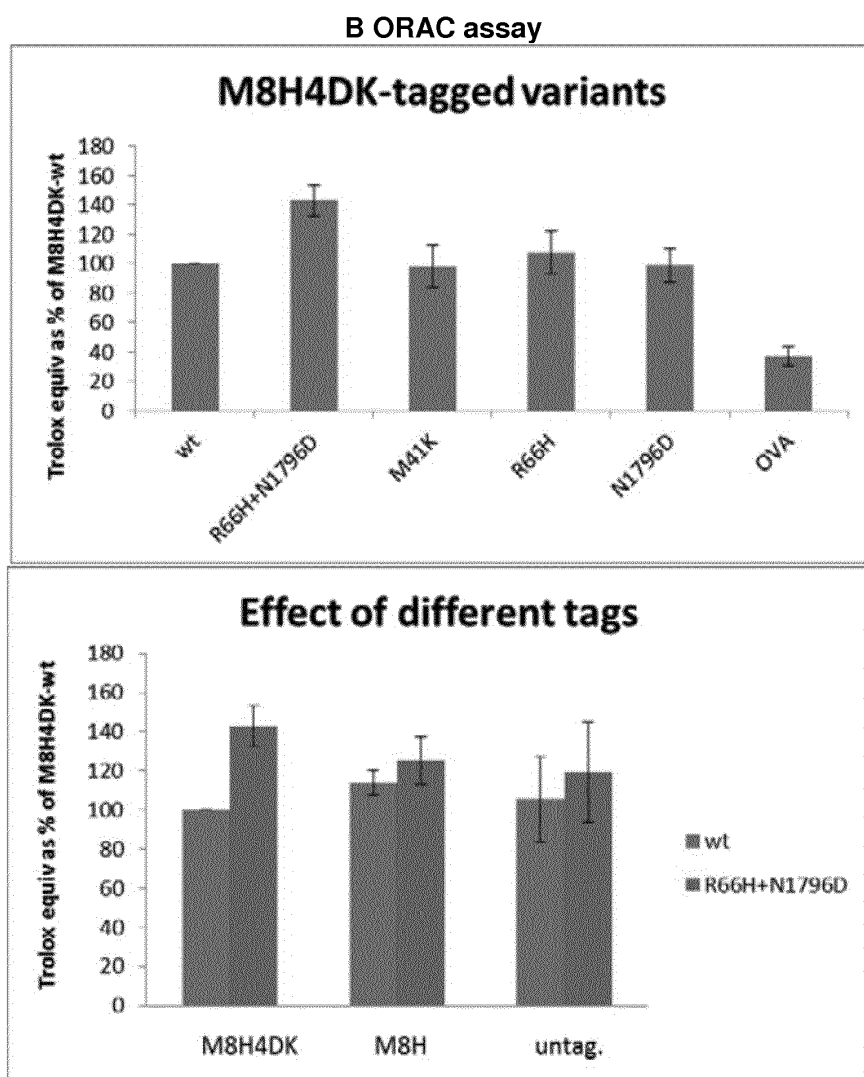


Figure 6

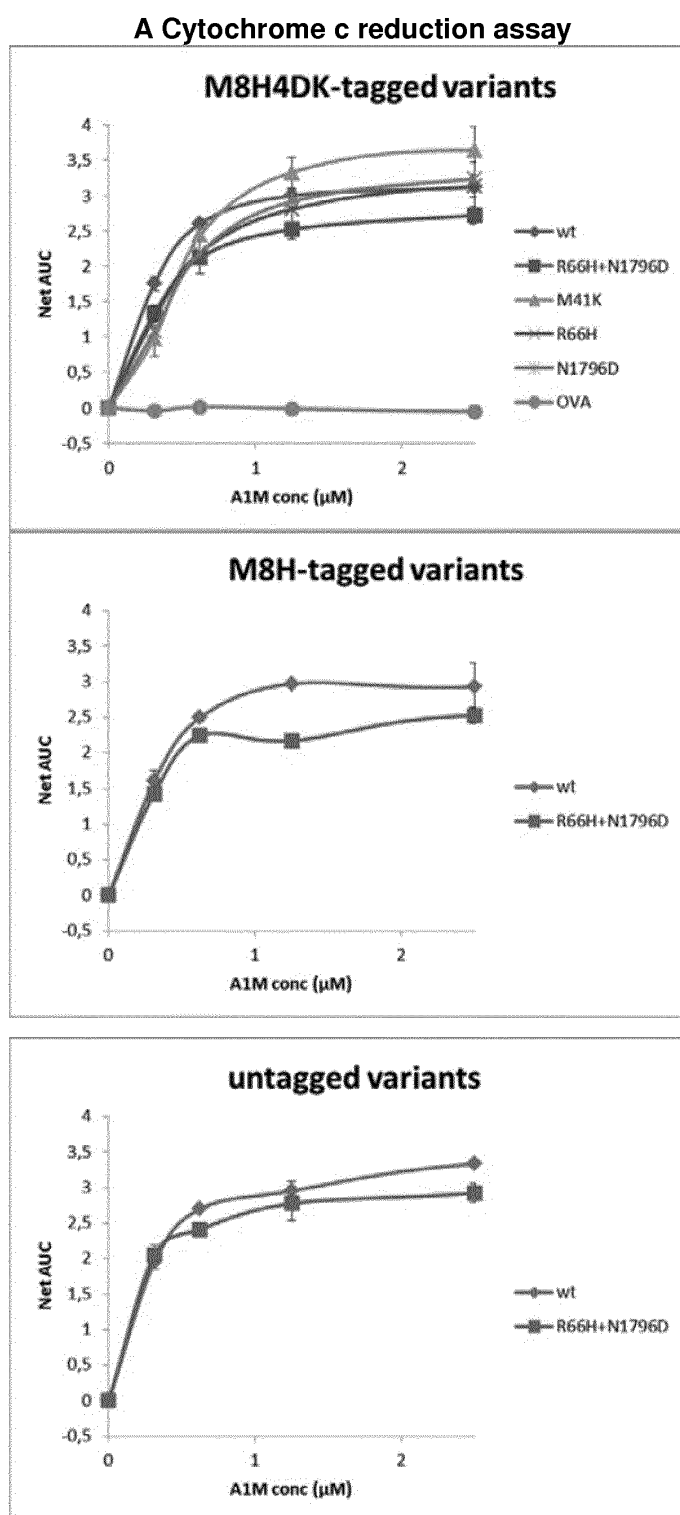
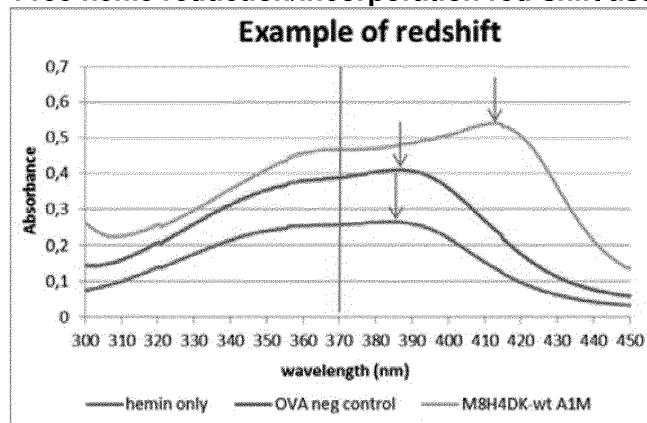


Figure 6 – continued

B Free heme reduction/incorporation red-shift assay

#	variant	max (370-450nm)	ratio 413:386
	heme only	386	0.52
	OVA	387	0.59
60	M8H4DK-wt (rhA1M)	413	1.13
35	M8H4DK-R66H+N17,96D	411	1.11
37	M8H4DK-M41K	415	1.27
38	M8H4DK-R66H	412	1.12
39	M8H4DK-N17,96D	413	1.15
40	M8H-wt	415	1.34
41	M8H-R66H+N17,96D	415	1.41
61	M-wt	386	0.87
42	MR66H+N1796D	387	0.76

C Heme-agarose binding

#	variant	μM bound/ μM protein
	OVA	0.008
60	M8H4DK-wt (rhA1M)	0.71
35	M8H4DK-R66H+N17,96D	0.72
37	M8H4DK-M41K	0.76
38	M8H4DK-R66H	0.66
39	M8H4DK-N17,96D	0.69
40	M8H-wt	0.68
41	M8H-R66H+N17,96D	0.72
61	M-wt	0.68
42	MR66H+N17,96D	0.66

Figure 7

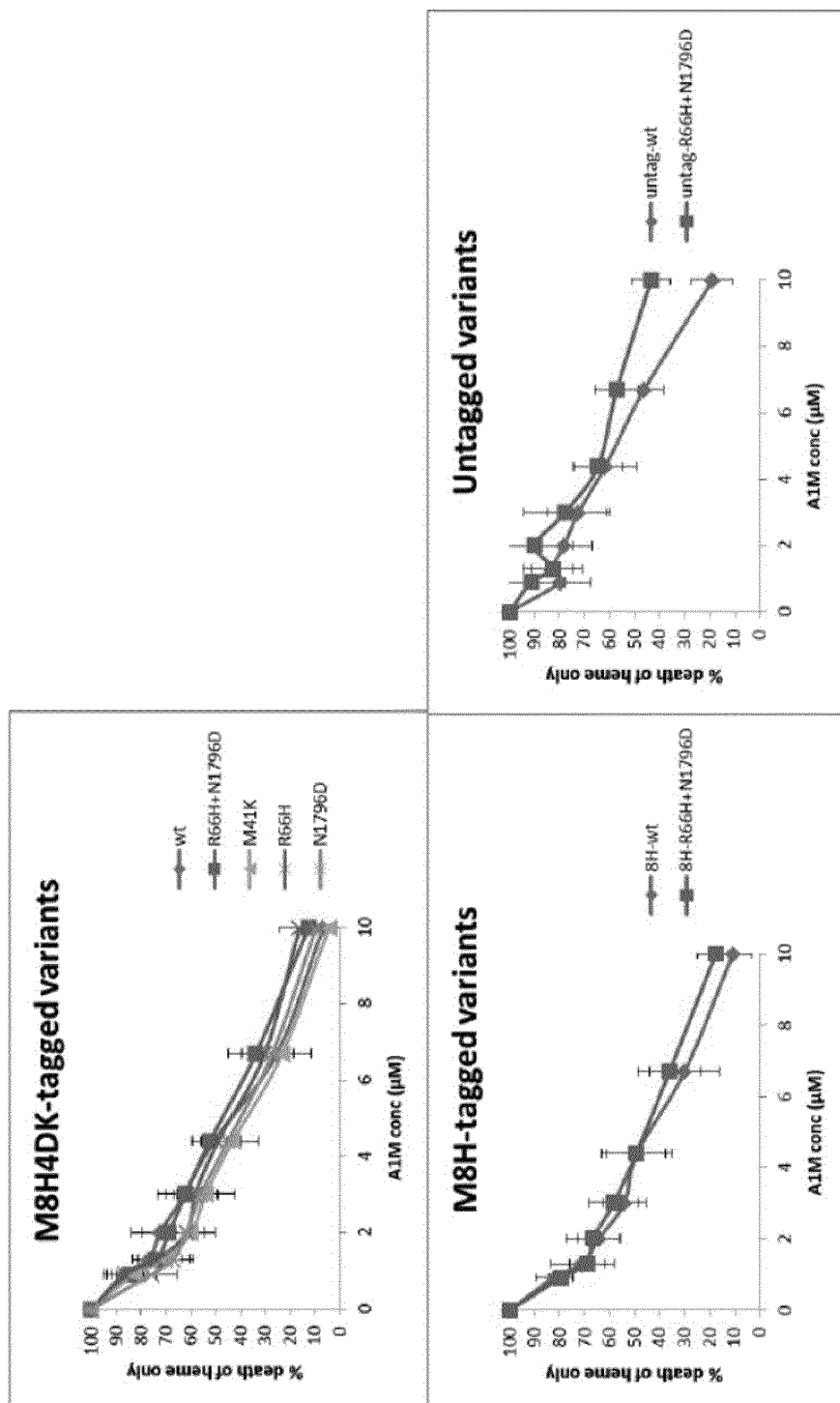


Figure 8

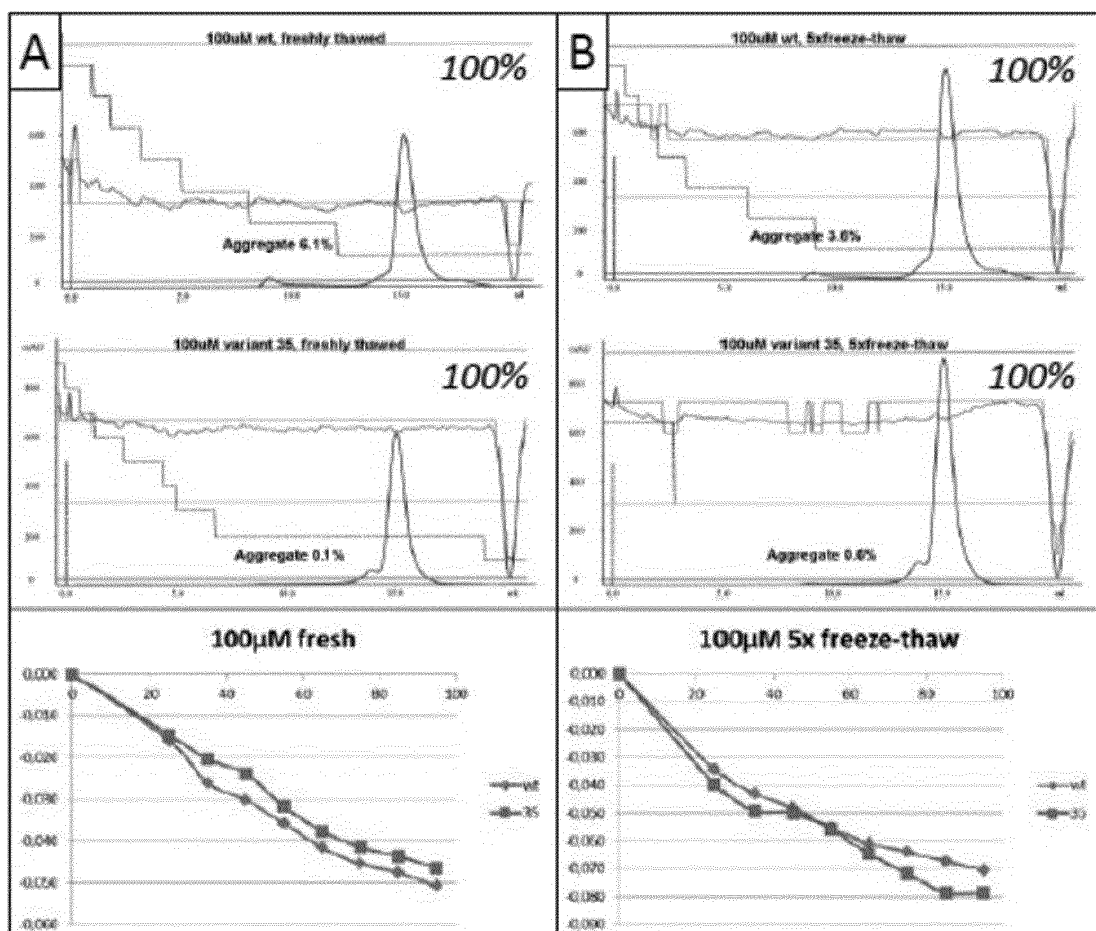


Figure 8 – continued

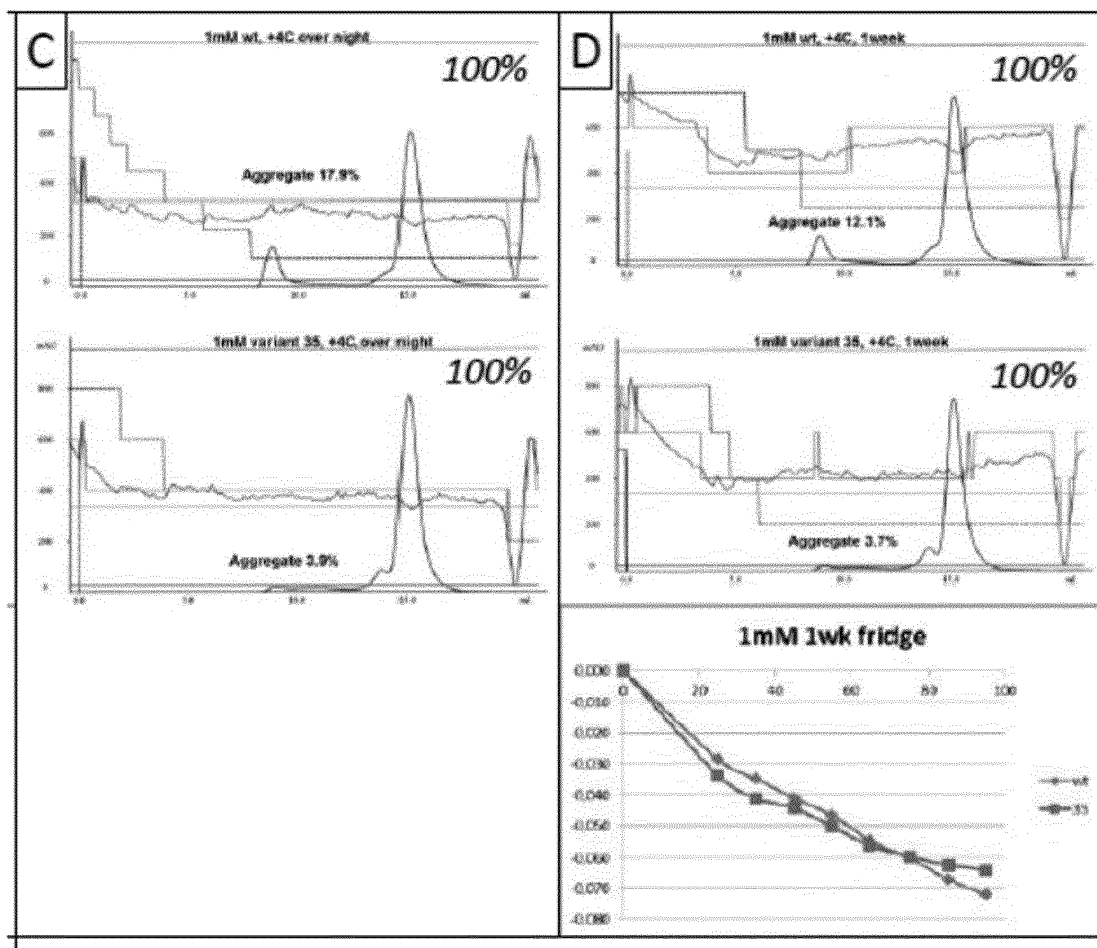


Figure 8 – continued

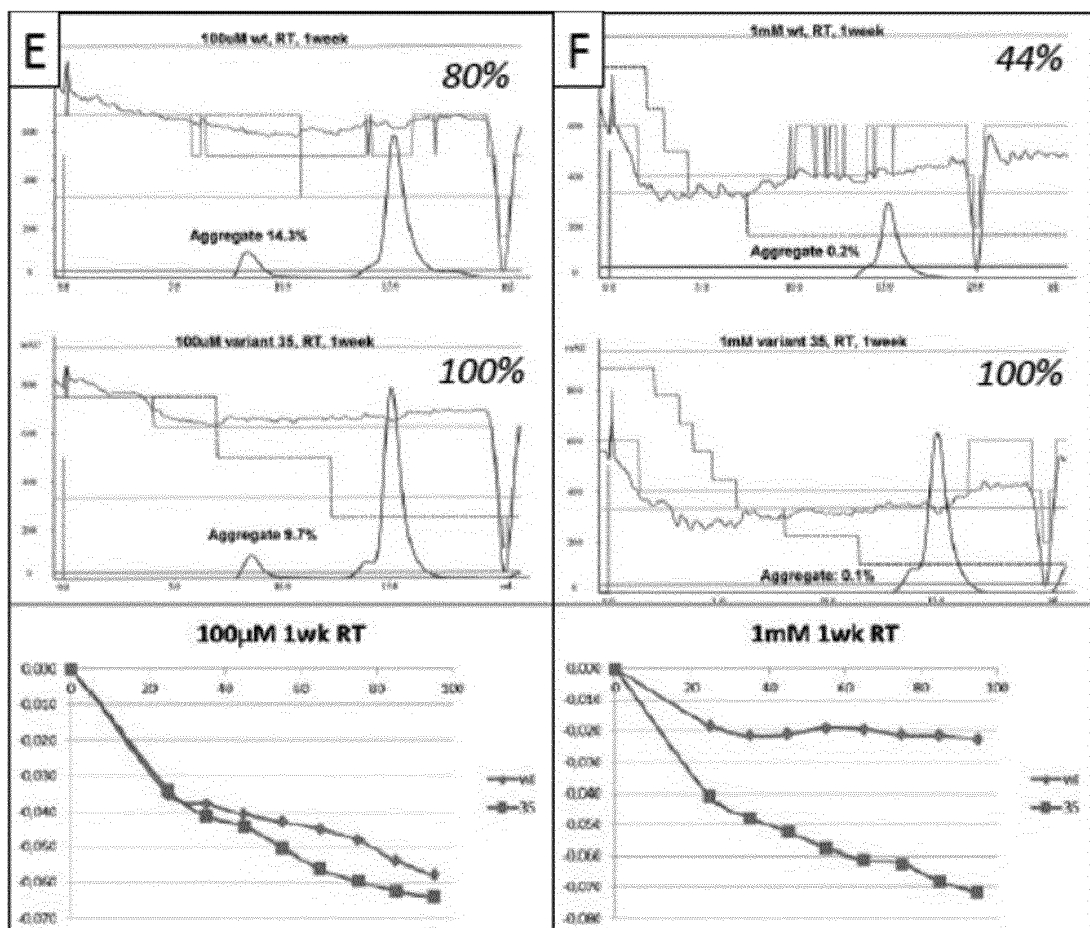


Figure 9

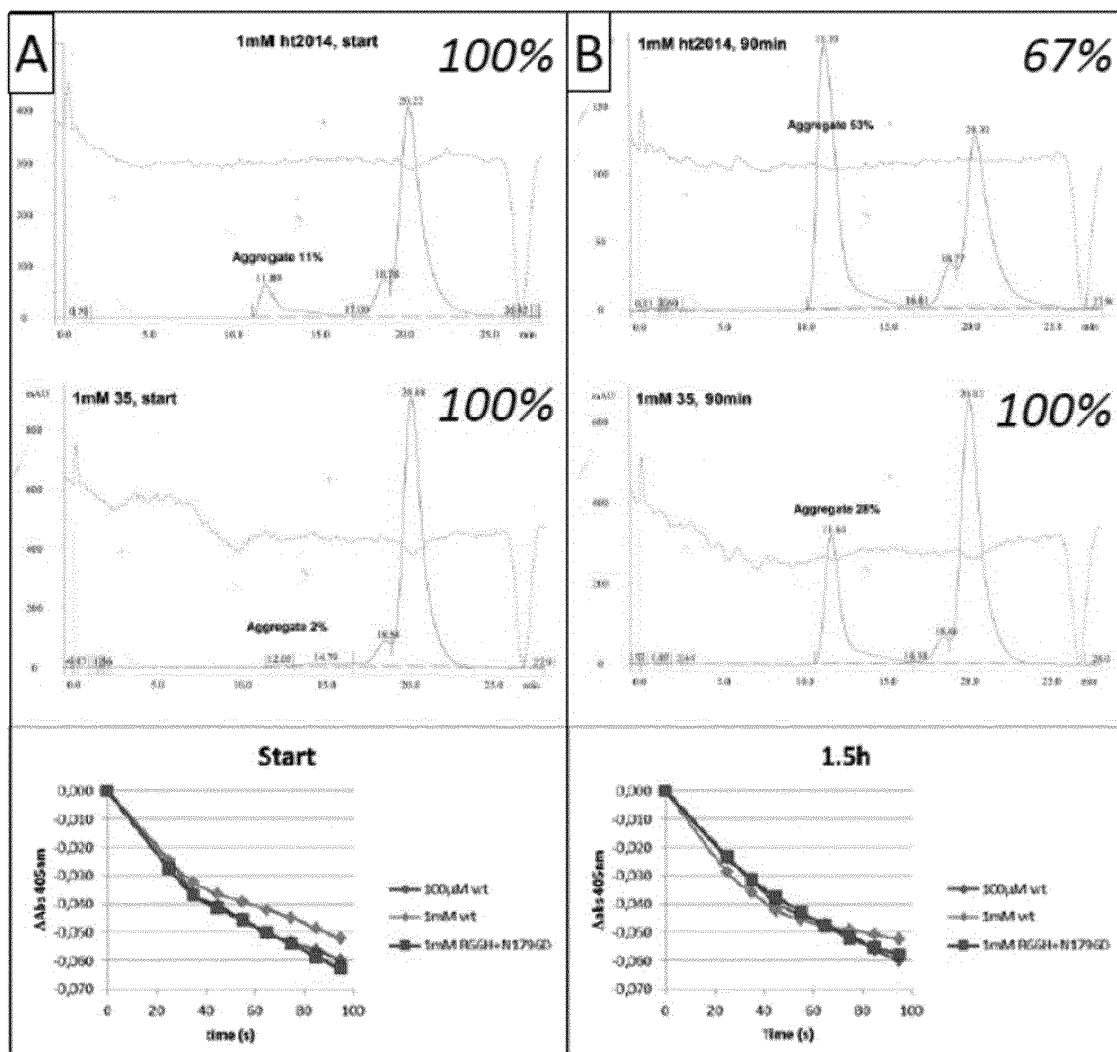


Figure 9 – continued

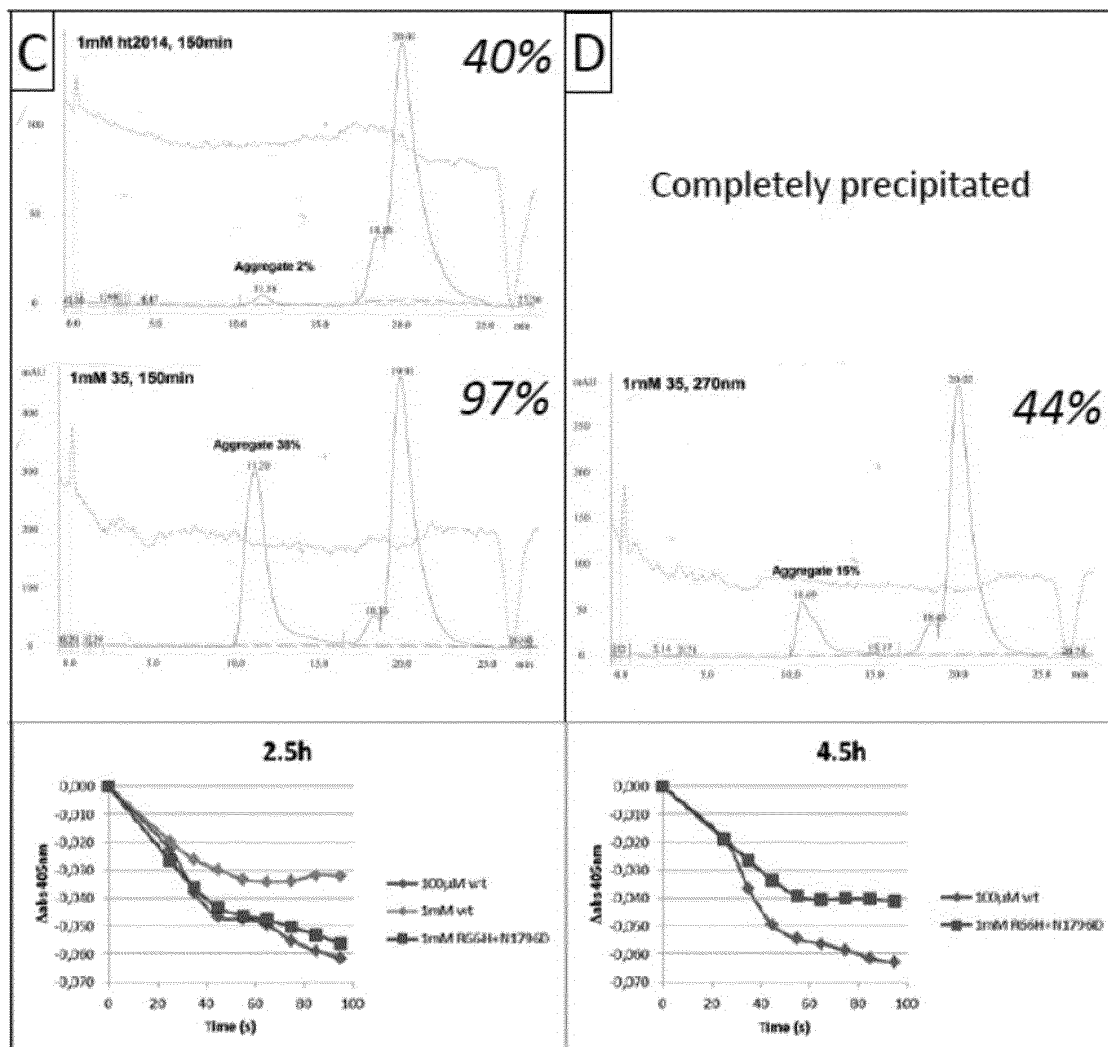


Figure 10

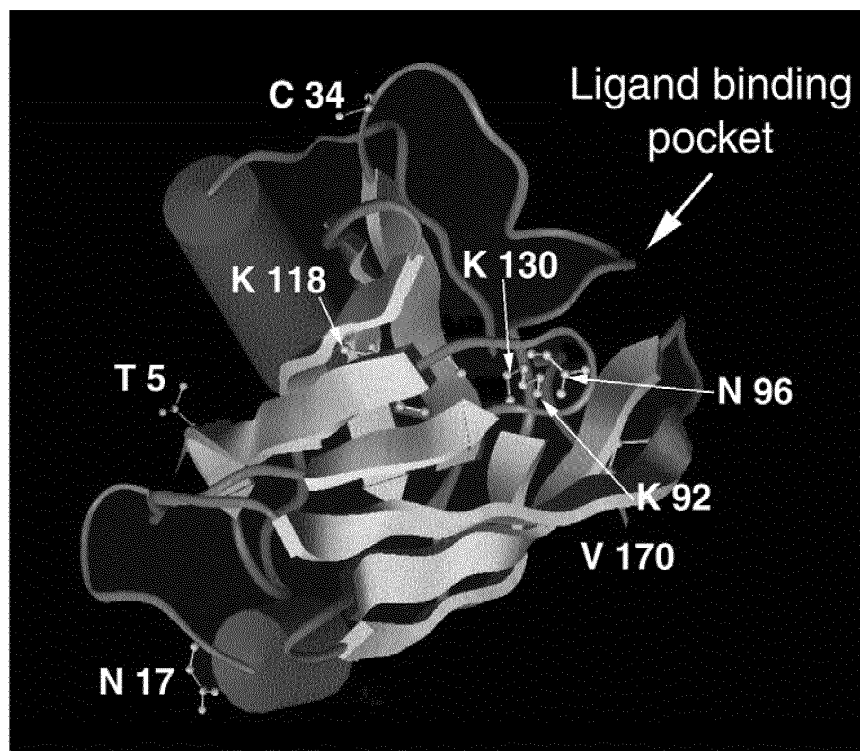


Figure 11

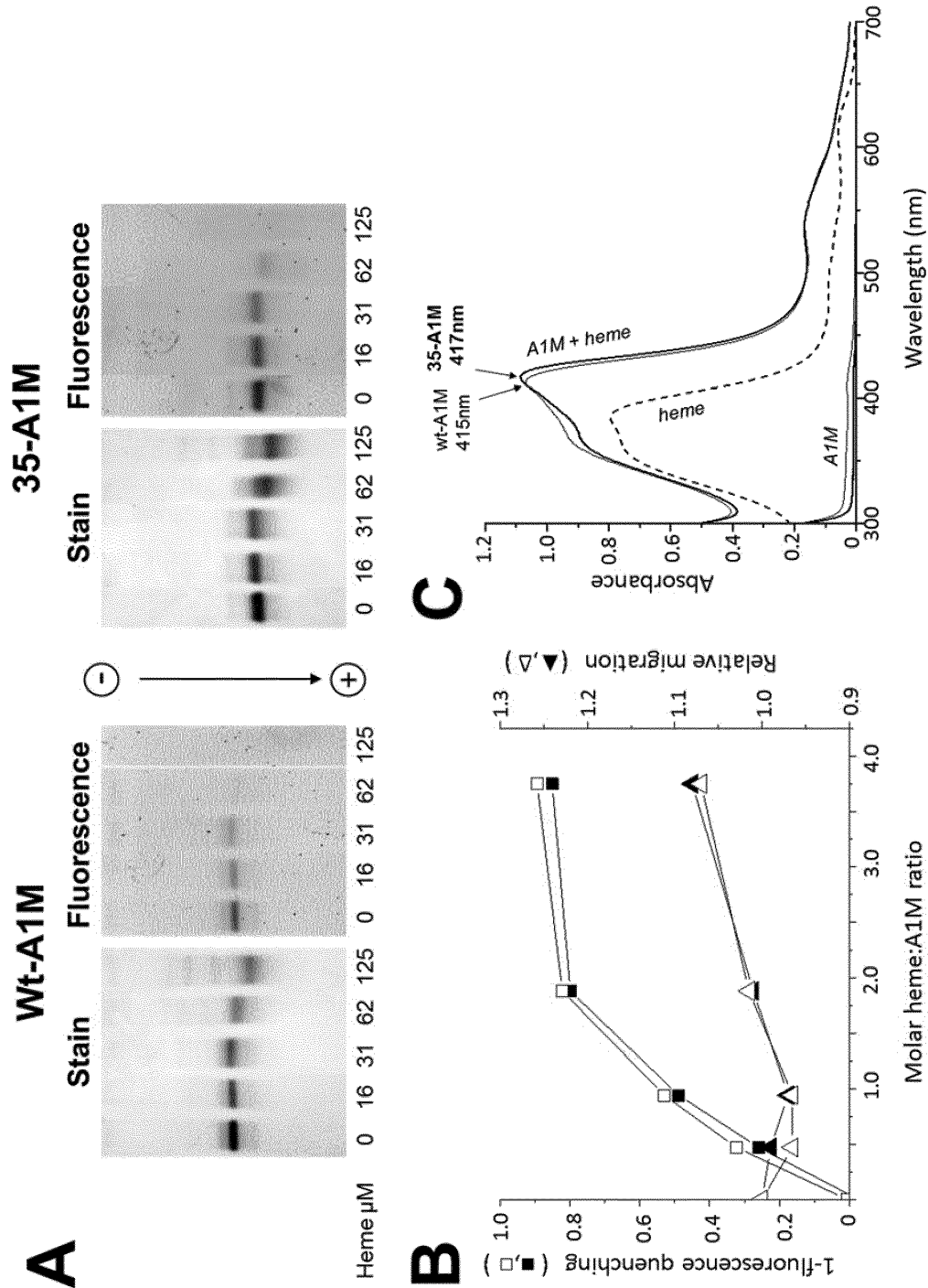


Figure 12

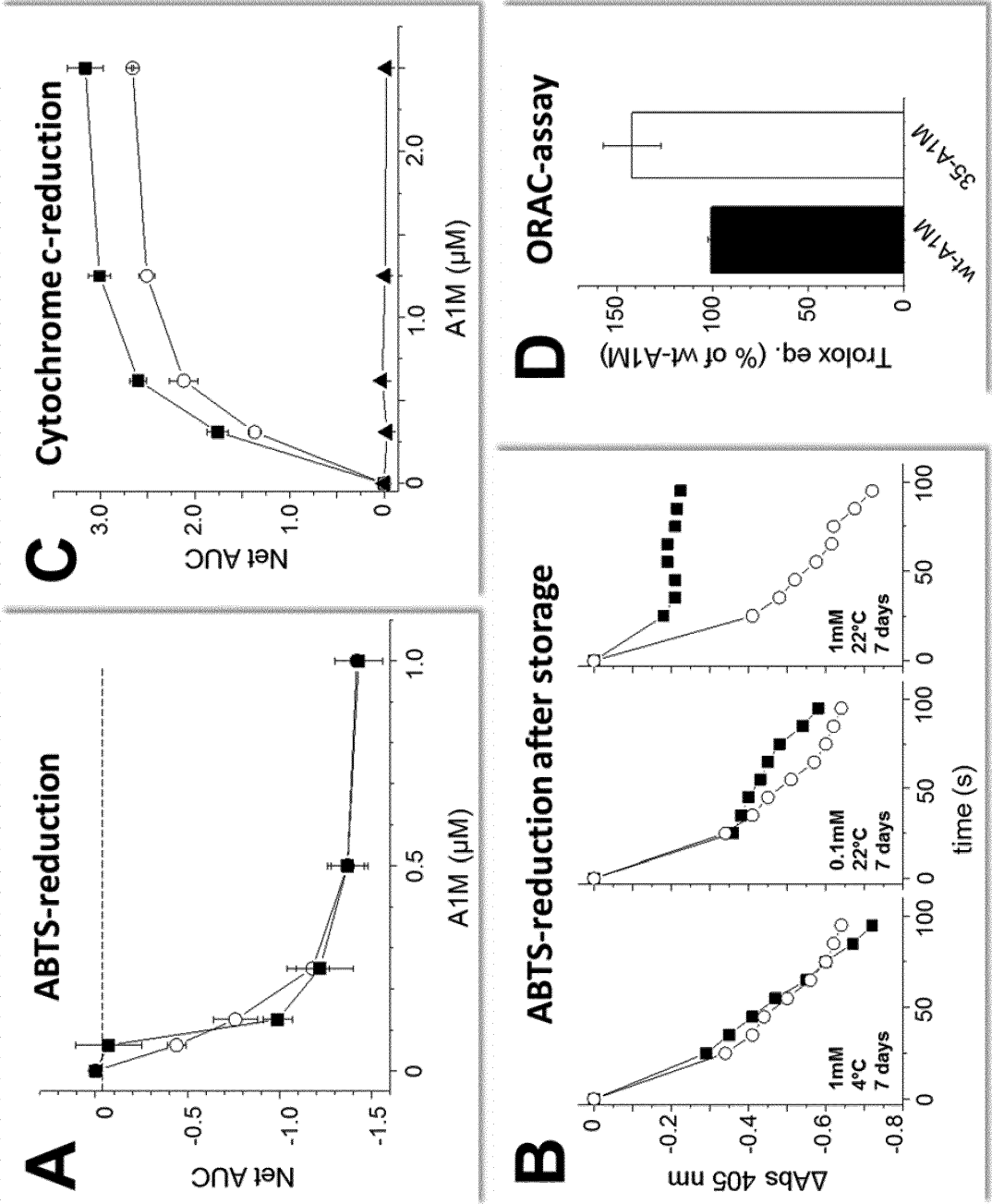


Figure 13

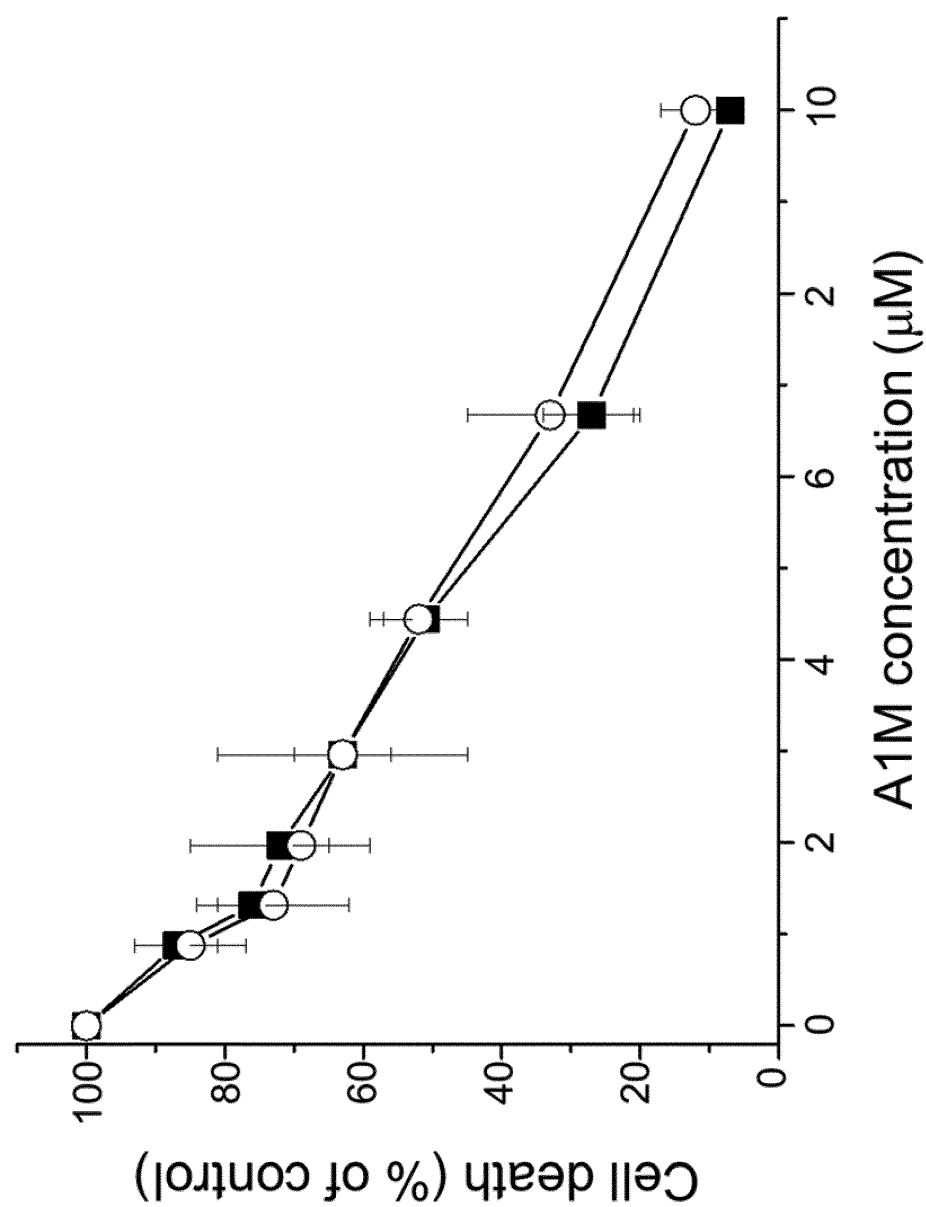


Figure 14

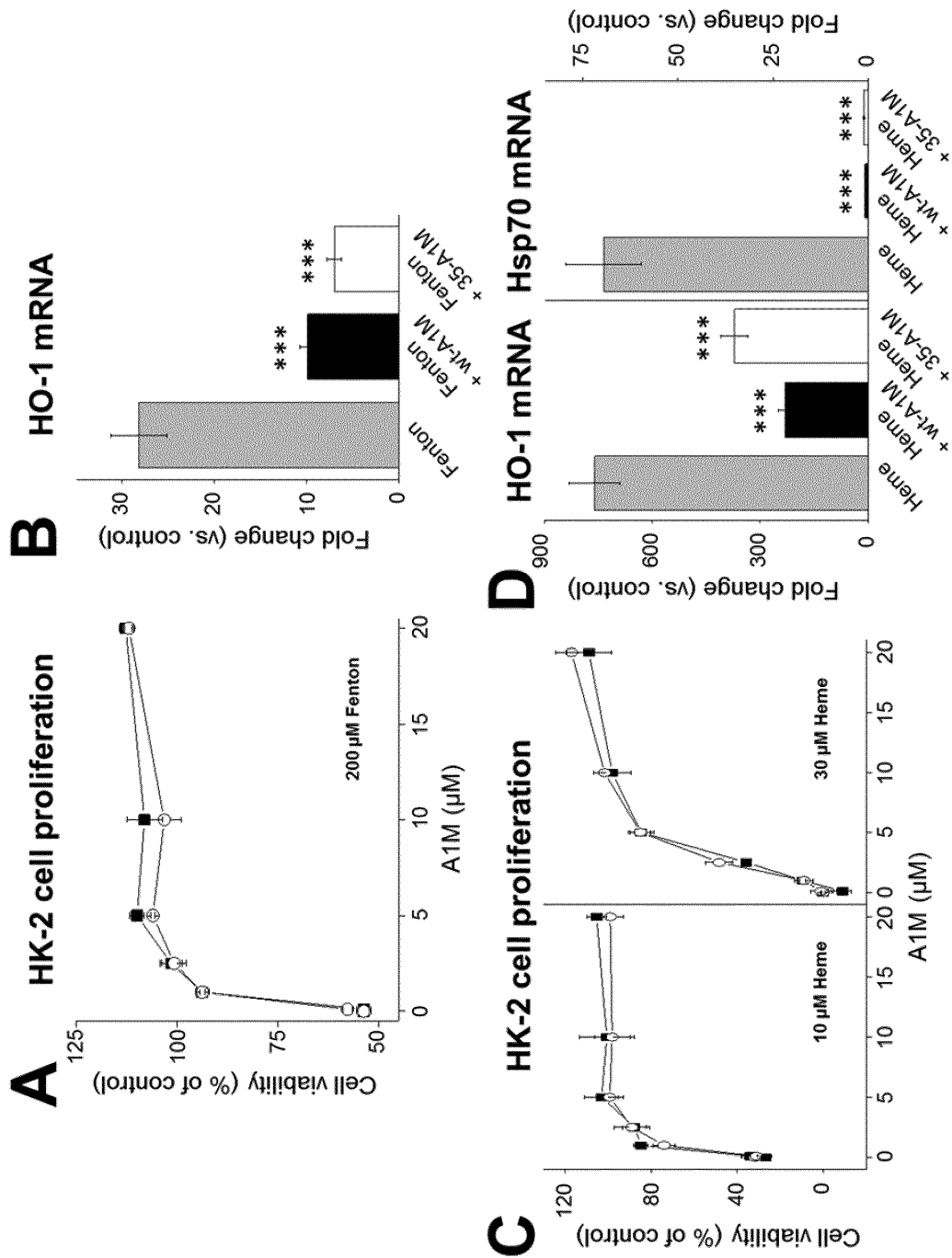


Figure 15

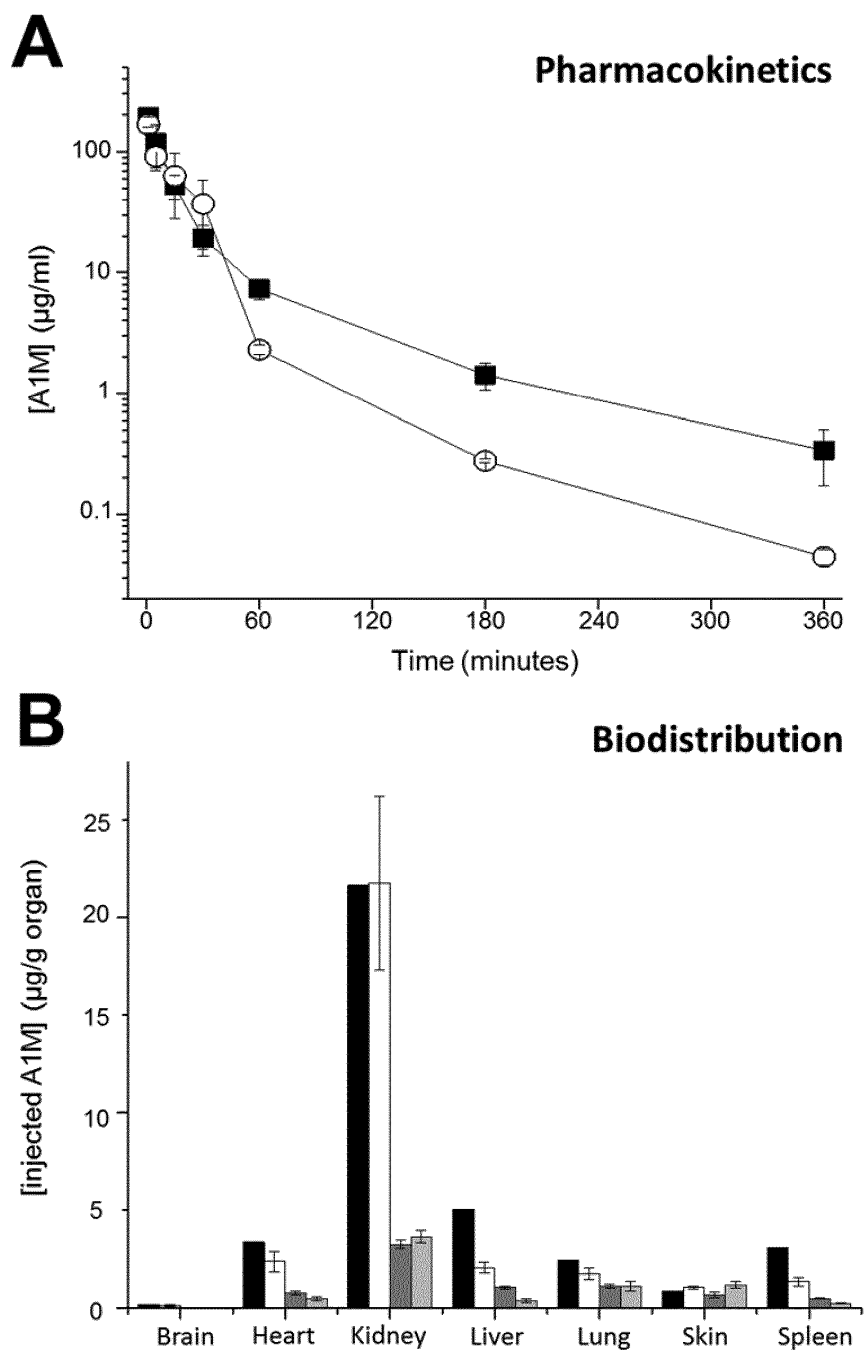


Figure 16

